USGS NSF GRIP Opportunity

USGS Center:	Michigan-Ohio Water Science Center
Project Title:	Development of methods distinguishing live and dead cell DNA for qPCR detection and quantification of pathogen genes in environmental samples for quantitative microbial risk assessment
Project Hypothesis or Objectives:	Molecular methods such as quantitative polymerase chain reaction (qPCR) allow scientists the ability to target specific genes allowing for a rapid, sensitive, and cost-effective way to determine the presence and relative abundance of potential pathogen genes in the environment. Although there are many benefits to qPCR such as not relying on the ability to culture fastidious microorganisms, one main limitation is the inability to differentiate between live and dead-cell DNA resulting in a potential overestimation of the abundance of pathogen genes. The ability to differentiate between live and dead cells would provide a more accurate understanding of risks associated with pathogens delivered from a broad array of sources including animal manure and human biosolids application and wildlife dissemination of zoonotic pathogens. Additionally, accurate estimations of pathogen genes in the environment are critical to understanding and evaluating the effectiveness of wastewater treatment plants, stormwater management, and green infrastructure on reducing human- and wildlife-health risks from combined sewer overflows (CSOs) and wastewater treatment plant effluent, stormwater runoff into neighboring surface waters, and storm drain effluent at recreational waterbodies. The propidium monoazide (PMA) approach was introduced recently with the aim of discriminating live-cell DNA from dead-cell DNA in downstream applications such as qPCR. PMA penetrates the damaged cell walls of dead pathogen cells and inhibits qPCR amplification allowing for a selective assessment of only the live pathogen cells. By correcting standard qPCR assays for detection of only viable pathogens, more accurate risk assessments can be conducted. Although PMA-qPCR has proven successful in the differentiation of live and dead cells in some matrices, additional method development and validation is required for challenging environmental applications. The Michigan Bacteriological Research Laboratory (MI-BaRL; USGS MI-OH Water Science Center) conducts research on the occurrence,

	source, abundance, and transport and fate of zoonotic pathogens in a variety of settings including both agricultural and urban environments, rivers, inland lakes, groundwater, and the Great Lakes. As part of ongoing research projects, the MI-BaRL develops and optimizes new molecular tools and assays, such as PMA methods for downstream qPCR analysis and quantitative microbial risk assessment (QMRA). Overarching project goals include 1) continuing to develop and optimize PMA-qPCR methods for zoonotic pathogens (including Campylobacter jejuni, Salmonella enterica, E.coli 0157:H7, and Shigella spp.); 2) determining the effectiveness of these methods in differentiating live and dead-cell DNA cells in various environmental matrices; 3) validating these methods in a variety of environmental matrices including water, sediment, manure, and algae; and 4) evaluating how environmental results can be used to inform QMRAs and gauge potential risks to human health. The development and optimization of PMA-qPCR methods and subsequent results combined with USGS capabilities in environmental and ecological assessment could be used by local, State, and other Federal agencies to evaluate potential risks via QMRA and to target limited resources on priority pathogens, sources, or processes to better manage environmental health.
Duration:	6-9 months (9 months preferable)
Internship Location:	Lansing, MI
Area of Discipline:	microbiology, environmental microbiology, public health, environmental health, environmental science, disease ecology
Expected Outcome:	The intern will work closely with USGS scientists on multiple research projects funded by the USGS, EPA, and other agencies. The intern will be expected to optimize assay conditions for 4 PMA-qPCR assays. qPCR assays may need to be optimized for PMA incorporation from previously developed qPCR assays used in MI-BaRL research studies. If current qPCR assays are not conducive to use with PMA, new assays will be developed. The intern will produce data from bench-scale examinations on the use of PMA-qPCR to detect live vs dead-cells. Assays will be developed using Taqman® qPCR and SYBR chemistry methods on a StepOne Real-Time PCR System (Life Technologies). Development of standard curves and calculations to determine gene copies numbers and development of spike and recovery for each PMA-qPCR method will be documented for future project use. The intern will be strongly encouraged to present results in a formal webinar at the study conclusion. Depending on the study outcomes, results may be published as a USGS report or peer-reviewed journal article with the intern as a contributing author. This project continues to develop tools and methods necessary to

	evaluate pathogens in the environment and enhances our ability to study the occurrence, survival, transport, and fate of zoonotic pathogens and in the environment. This project addresses current method limitations and attempts to resolve these limitations in order to further our understanding of pathogens in the environment and risk to human and wildlife health. This project contributes to ongoing collaborative research with the USGS Toxic Substances Hydrology Program's Contaminants of Emerging Concern project and addresses multiple goals outlined in the USGS Environmental Health science strategy by improving methods for detecting pathogens in the environment and examining the relative contributions of different sources of pathogenic bacteria that could lead to adverse human and animal health outcomes.
Special skills/training Required:	The intern will need a background of three or more years of microbiology education or with at least one year of independent qPCR experience. Basic microbiological culturing experience and aseptic technique are essential. The intern should have experience designing and optimizing qPCR assays, interpreting qPCR analysis and maintaining lab QA/QC. Please note, laboratory work may involve prolonged standing in a laboratory environment. Work in the laboratory may involve use of equipment which can result in exposure to dust, chemicals, mechanical and laboratory hazards, and noise. Special safety precautions are required including use of gloves, coats, etc.
Duties/Responsibilities:	The intern will work with microbiologists at the MI-BaRL to develop and optimize PMA-qPCR methods for detecting and quantifying zoonotic pathogens in multiple environmental matrices. The intern will work with MI-BaRL project leads to develop a work plan identifying project objectives and goals and outlining an experimental approach that addresses the project goals previously listed. The intern will be an integral part of the project with the end goal of developing validated and publishable PMA-qPCR experimental protocols for several zoonotic pathogens. Responsibilities would likely include testing various PMA experimental parameters such as the length of dark and light incubation, PMA concentration, ancillary qPCR conditions, evaluation of these results, and determination of the optimal PMA experimental parameters for discriminating live- and dead-cell DNA for specific target pathogen(s). Once the PMA method is optimized, the intern will work to validate this method in environmental samples. The intern will also evaluate the effectiveness of these methods using bench scale pathogen experiments. These methods and risk modelling will be incorporated into ongoing research studies and future study development. The intern will also participate in MI-BaRL lab discussions and program development discussions focused on identifying understudied and emerging zoonotic pathogens for subsequent assay development.

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